AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims

Claims 1 – 22 (cancelled)

Claim 23 (currently amended): A method for removing endotoxin from a plasmid DNA solution comprising:

a) filtering a solution comprising plasmid DNA through a series of filters including ~~at least one~~ **a** glass fiber filter and ~~at least one~~ **a** nylon filter;

b) contacting the solution comprising plasmid DNA with a trimethylamino ethyl (TMAE) anion exchange chromatography resin, the solution having a conductivity at which the plasmid DNA is bound to the resin; washing the resin to elute endotoxin; and eluting the plasmid DNA with a step or continuous gradient of increasing conductivity.

Claim 24 (previously presented): The method of claim 23, wherein the TMAE anion exchange chromatography resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group.

Claim 25 (previously presented): The method of claim 23, wherein the plasmid DNA solution is loaded on the resin in a solution having a conductivity of less than about 50 mS/cm.

Claim 26 (previously presented): The method of claim 25, wherein the plasmid DNA is step eluted with a series of buffers of increasing conductivity in a range of from about 50 to about 90 mS/cm.

Claim 27 (cancelled)

Claim 28 (previously presented): The method of claim 23, where the plasmid DNA solution is filtered through the series of filters prior to contacting the plasmid DNA solution with the anion exchange chromatography resin.

Claim 29 (previously presented): The method of claim 23, wherein the plasmid DNA solution is a clarified lysate obtained after alkaline lysis of bacterial cells comprising the plasmid DNA and removal of precipitated proteins, chromosomal DNA and cell debris.

Claim 30 (previously presented): The method of claim 29, wherein the clarified lysate is further neutralized to a pH of about 7 to about 8.5.

Claim 31 (previously presented): The method of claim 30, wherein the clarified lysate is further neutralized with a buffer that decreases an ionic strength of the lysate for direct loading onto the anion exchange resin.

Claim 32 (previously presented): The method of claim 30, wherein the lysate is neutralized with a buffer that comprises Tris base.

Claim 33 (currently amended): A method for removal of endotoxin from a plasmid DNA solution comprising:

a) filtering the plasmid DNA solution through a series of filters comprising ~~at least one~~ **a** glass fiber filter and ~~at least one~~ **a** nylon filter;

b) loading the filtered plasmid DNA solution onto a column comprising trimethylamino ethyl (TMAE) anion exchange resin, wherein the plasmid DNA solution is loaded onto the column in a loading buffer having a conductivity below which the plasmid DNA would elute from the resin; washing the column with a buffer having a conductivity sufficient to elute endotoxin but not plasmid DNA from the resin; and eluting the plasmid DNA with a step or continuous gradient of increasing conductivity, thereby producing a solution of anion exchange purified plasmid DNA

c) filtering the solution of anion exchange purified plasmid DNA through a further series of filters comprising ~~at least one~~ **a** glass fiber filter and ~~at least one~~ **a** nylon filter to remove residual endotoxins.

Claim 34 (previously presented): The method of claim 33, wherein the plasmid DNA solution comprises a clarified lysate obtained following alkaline lysis and precipitation using continuous flow static mixers.

Claim 35 (previously presented): The method of claim 34, wherein the clarified lysate is neutralized to a pH of about 7 to about 8.5 prior to anion exchange chromatography.

Claim 36 (previously presented): The method of claim 35, wherein the clarified lysate is neutralized with a buffer that deceases an ionic strength of the lysate for direct loading onto the anion exchange resin.

Claim 37 (currently amended): A pharmaceutical scale method for purifying plasmid DNA comprising:

a) mixing a solution of bacterial cells comprising the plasmid DNA with an alkaline lysis solution by flowing through a first static mixer to obtain a lysate;
b) contacting the lysate with a potassium acetate precipitation solution by flowing through a second static mixer, thereby forming a precipitation mixture;
c) removing a precipitate from the precipitation mixture thereby forming a clarified lysate;
d) filtering the clarified lysate through a series of filters comprising ~~at least one~~ **a** glass filter and ~~at least one~~ **a** nylon filter thereby forming a filtered lysate;
e) loading the filtered lysate onto a trimethylamino ethyl (TMAE) anion ion exchange chromatography resin under conditions wherein the plasmid DNA is retained on the resin, washing the resin with a buffer that removes weakly bound impurities from the resin, and eluting the plasmid DNA with a step or continuous saline gradient, thereby producing a solution of anion exchange purified plasmid DNA; and
f) filtering the solution of anion exchange purified plasmid DNA through a further a series of filters comprising at least one glass filter and at least one nylon filter to further remove residual endotoxins.

Claim 38 (previously presented): The method of claim 37, further comprising a step of RNase digestion.

Claim 39 (previously presented): The method of claim 37, further comprising a step of adjusting the pH and conductivity of either the precipitation mixture or the clarified lysate to a pH in the range of about 7 to about 8.5 and a conductivity of less than about 50mS/cm prior to the filtering step wherein the filtered lysate can be directly loaded onto the anion ion exchange chromatography resin.

Claim 40 (previously presented): The method of claim 37, wherein the trimethylamino ethyl (TMAE) anion ion exchange resin comprises a methacrylate based copolymer having a tentacle linked TMAE functional group.

Claim 41 (previously presented): The method of claim 37, further comprising the step of purifying the plasmid DNA solution using ultrafiltration in the presence of a gel layer that is allowed to form before starting ultrafiltration.

Claim 42 (previously presented): The method of claim 41, wherein the ultrafiltration unit is an open channel tangential flow ultrafiltration unit.

Claim 43 (currently amended): A method for purifying plasmid DNA comprising:

a) lysing the bacterial cells by alkaline lysis and precipitation through a series of continuous flow static mixers to provide a lysate;
b) clarifying the lysate and adjusting the pH and conductivity of the lysate to a pH of about 7.0 to about 8.5 and a conductivity of less than about 50mS/cm;
c) filtering the clarified and adjusted lysate through a filter series comprising ~~at least one~~ **a** glass filter and ~~at least one~~ **a** nylon filter to provide a filtered lysate;
d) purifying the filtered lysate by anion exchange chromatography using a methacrylate based copolymer resin having a tentacle linked TMAE functional group to provide a purified plasmid DNA solution;
e) filtering the purified plasmid DNA solution through a further filter series comprising a glass filter and a nylon filter to reduce endotoxin levels; and
(f) optionally, ultrafiltering and diafiltering the anion exchange purified plasmid DNA through a tangential flow open channel device in the presence of a gel-layer that is formed by an initial period of recirculation.

Claim 44 (previously presented): The method of claim 23, wherein the nylon filter is a N66 nylon filter.

Claim 45 (previously presented): The method of claim 33, wherein the nylon filter is a N66 nylon filter.

Claim 46 (previously presented): The method of claim 37, wherein the nylon filter is a N66 nylon filter.

Claim 47 (previously presented): The method of claim 43, wherein the nylon filter is a N66 nylon filter.